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Autophagy is not uniformly cytoprotective: a personalized medicine approach for autophagy inhibition as a therapeutic strategy in non-small cell lung cancer

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Abstract

BACKGROUND—Lung cancer is the leading cause of cancer-related death worldwide. In addition to surgical resection, which is considered first-line treatment at early stages of the disease, chemotherapy and radiation are widely used when the disease is advanced. Of multiple responses that may occur in the tumor cells in response to cancer therapy, the functional importance of autophagy remains equivocal; this is likely to restrict current efforts to sensitize this malignancy to chemotherapy and/or radiation by pharmacological interference with the autophagic response.

SCOPE OF REVIEW—In this review, we attempt to summarize the current state of knowledge based on studies that evaluated the function of autophagy in non-small cell lung cancer (NSCLC) cells in response to radiation and the most commonly used chemotherapeutic agents.

MAJOR CONCLUSIONS—In addition to the expected prosurvival function of autophagy, where autophagy inhibition enhances the response to therapy, autophagy appears also to have a “non-cytoprotective” function, where autophagy blockade does not affect cell viability, clonogenicity or tumor volume in response to therapy. In other cases, autophagy may actually mediate drug action via expression of its cytotoxic function.

GENERAL SIGNIFICANCE—These observations emphasize the complexity of autophagy function when examined in different tumor cell lines and in response to different chemotherapeutic agents. A more in-depth understanding of the conditions that promote the unique functions of autophagy is required in order to translate preclinical findings of autophagy inhibition to the clinic for the purpose of improving patient response to chemotherapy and radiation.

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1. Introduction

Lung cancer is one of the most common malignancies affecting both men and women in the United States¹. Of its multiple subtypes, non-small cell lung cancer (NSCLC) accounts for nearly 85% of the diagnosed cases^{2, 3}. The American Cancer Society estimates that new lung cancer cases in the United States for 2015 are likely to approach 115,610 in men and almost 105,590 in women⁴. Despite a decrease in death rates for cancer overall, lung cancer remains the leading cause of cancer-related mortality, accounting for 27% of all cancer deaths⁵.

While surgical resection of lung tumors is the optimal therapeutic approach when the disease is diagnosed at an early stage, unfortunately most patients present with advanced, inoperable disease since lung cancer is largely asymptomatic during its early stages. In patients with overexpression of the epidermal growth factor receptor (EGFR), which involves approximately 10–15% of the population, a number of new drug therapies have demonstrated various degrees of success⁶. In this context, a number of other targeted therapies are under investigation⁷ with the goal of developing more individualized approaches based on both molecular and histological characterization⁸. Of these novel therapies, drugs that target the Anaplastic Lymphoma Kinase (ALK) have shown significant reductions in tumor volume or maintenance of a stable disease state in a subset of lung cancer patients with rearrangements in the ALK gene; such fusion lesions render cells dependent on ALK signaling and susceptible to the actions of tyrosine kinase inhibitors⁹. In almost all cases, ALK genetic rearrangements have been linked to mutations in other oncogenes such as EGFR and k-RAS¹⁰ and, interestingly, in patients who are mostly young and with low or no history of tobacco consumption⁹. In addition, several immunotherapies have also been developed as second-line treatment for advanced cases that fail to respond to platinum therapy. Nivolumab, an anti PD-1 receptor monoclonal antibody, was approved by the FDA in 2014 and has shown acceptable clinical efficacy in patients with terminal squamous cell carcinoma¹¹ and overall survival superiority over other checkpoint inhibitors¹².

Despite these considerable advancements in targeted therapy of lung cancer, the primary therapeutic strategy for most patients with advanced disease remains radiation therapy in combination with cytotoxic chemotherapy, i.e. chemoradiation⁸. The primary drugs currently used in chemoradiation are the DNA cross linking agents cisplatin and carboplatin, the microtubule poisons paclitaxel and docetaxel and the DNA topoisomerase II inhibitor etoposide¹³. Many of these drugs have significant limitations, including adverse effects, decreased quality of life and drug resistance^{14–16}; consequently, there have been continuing efforts to increase sensitivity of non-small cell lung cancer to these therapeutics.

Autophagy has historically been considered as a defensive mechanism that is observed under conditions of cellular stress, responsible for metabolic compensation and organelle turnover¹⁷. However, multiple other functions of autophagy have been characterized in different cancer cell lines, where it can play an anti-survival role or act as a cellular mechanism mediating sensitization to therapy¹⁸.

Many of the chemotherapeutic agents that are commonly utilized in the treatment of non-small cell lung cancer have been shown to induce autophagy^{19–21}. Autophagy promotion in response to these stimuli can be detected by a number of classical methods that include increased lipidation of LC3B (conversion of LC3B form I to form II), p62/SQSTM1 degradation, fluorescent microscopy, flow cytometry and the use of acidotropic dyes^{22, 23}.

It has been widely argued that inhibition of chemotherapy-induced autophagy could serve as a useful strategy for the treatment of drug and radiation resistant cancers²⁴. A host of clinical trials are currently in progress evaluating the use of autophagy inhibitors, specifically chloroquine and hydroxychloroquine, in combination with the conventional chemotherapeutics such as platinum-based compounds, taxanes and EGFR inhibitors (www.clinicaltrials.gov). However, we would argue, based on the literature to be discussed below, that the impact of autophagy inhibition on the response to chemotherapy and radiation is likely to be inconsistent and largely dependent on the genetic background of the tumor cell as well as the nature of the chemotherapeutic agent. More specifically, it has become evident that there are many cases where autophagy inhibition does not result in sensitization to the autophagy-inducing stress (drugs or radiation), findings that have led us to identify a form of autophagy that we have termed “*non-cytoprotective*”^{25–27}. The clinical challenge will be to identify biomarkers that can quickly distinguish the cytoprotective form of autophagy from the other functional forms so as to facilitate the selection of those patients who are most likely to benefit from the therapeutic strategy of autophagy inhibition (i.e. a personalized medicine approach as opposed to combining autophagy inhibition with chemotherapy or radiation in all patients presenting with lung cancer).

2. Chemotherapy-induced autophagy

Cisplatin and carboplatin

Platinum-based compounds are considered first line drugs in the treatment of advanced non-small cell lung cancer (www.cancer.gov). Cisplatin exerts its cytotoxic effect via the production of DNA intra- and interstrand crosslinks¹⁵. The resulting damage to DNA is recognized by a variety of proteins involved in DNA repair that drive the cell into arrest or apoptosis in a dose-dependent manner¹⁵. In studies in the H460 NSCLC cell line, a range of cisplatin concentrations ranging between 5 μ M and 20 μ M were shown to induce an autophagic response¹⁹. Autophagy was identified by increased LC3B conversion from form I to form II, in addition to microscopic evaluation of morphological changes in the cells. In this study, autophagy was found to be cytoprotective, based on the MTT assay comparing cell viability between cell populations treated with cisplatin alone and populations where cisplatin was combined with 3-methyladenine (3-MA), a class III phosphoinositide 3-kinase (PI3K) inhibitor that blocks the formation of autophagosomes. The combination clearly promoted enhanced antitumor sensitivity and increased apoptosis based on Annexin V/PI staining and FACS analysis. However, no studies were performed where autophagy was inhibited by genetic silencing strategies. Furthermore, as 3-MA may have non-specific effects in addition to potentially being toxic to the cell at high concentrations, the absence of data for effects of 3-MA alone on cell viability or apoptosis makes it difficult to discern whether sensitization was occurring solely via inhibition of autophagy.

A study in which cisplatin-resistant A549 cells were exposed to cisplatin showed enhanced autophagy and elevated expression of high mobility group one box 1 protein (HMGB1), a highly conserved protein in many cancers that is sometimes considered to be a marker of autophagy²⁸. Silencing of HMGB1 by transfection with shRNA resulted in a reduction in autophagy (based mainly on LC3 status) and higher sensitivity to cisplatin as well as an increase in apoptosis. This report suggests that interference with autophagy could sensitize A549 cells that had acquired resistance to cisplatin. However, as was the case with the previous study, direct genetic or pharmacologic inhibition of autophagy was not performed, and while HMGB1 appears to play a role in autophagy induction, it is still not a widely accepted marker of autophagy.

Several other recent reports support the involvement of autophagy in the development of resistance to chemotherapy^{29–32}. Studies have shown that cisplatin-resistant cells express higher levels of Beclin-1 (the mammalian counterpart of yeast ATG6, a protein involved in the regulation of autophagy that also serves as a crossroad at which autophagy and apoptosis interact³³), increased conversion of LC3 from form I to form II and a decrease in p62/SQSTM1 levels, all indicative of increased induction of autophagy^{29–31}. It is frequently observed that upon autophagy blockade, for example by chloroquine (usually used in doses that are not cytotoxic but sufficient to block autophagy), a decrease in cisplatin-resistance is observed based on IC50 values and/or increased annexin V staining indicative of enhanced apoptosis²⁹. The sensitizing effect of chloroquine is clearly more pronounced in cisplatin-resistant A549 cells in comparison to the wild-type, cisplatin-sensitive A549 cells²⁹. These findings suggest that autophagy inhibition might prove to be most effective as a therapeutic strategy after the tumors develop resistance to platinum following multiple cycles of treatment (as the resistant cells were selected by continuous exposure to increasing concentrations of drug).

In general, as indicated above, cisplatin is often considered to initiate a cytoprotective autophagy that results in sensitization when blocked pharmacologically. However, this response to cisplatin is not necessarily consistent. Studies by *Sirichanchuen et al*³⁴ involving repetitive exposure to cisplatin in H460 cells to promote cisplatin resistance reported a *reduced* formation of autophagosomes in response to cisplatin, as indicated by significantly lower levels of LC3 in cisplatin-resistant cells, findings that were supported by assessment of acridine orange staining and FACS analysis. Here, it was the *induction* of autophagy using trifluoperazine³⁵ rather than autophagy inhibition that resulted in a reversal of the resistance to cisplatin and an increase in cisplatin-induced cytotoxicity. Consequently, in this experimental system, autophagy assumed a *cytotoxic role*. The induction of autophagy in the cisplatin-sensitive H460 cells was also associated with a small (20–30%) but significant increase in cisplatin sensitivity.

In contrast to the studies cited above in support of the cytoprotective function of cisplatin-induced autophagy, work by another team of investigators demonstrated that inhibition of autophagy using either 3-MA or silencing of the autophagy gene, *ATG5*, did not significantly alter the influence of cisplatin on viability of H460 cells, indicative of a *non-cytoprotective* function of autophagy³⁶. Furthermore, when autophagy was inhibited either pharmacologically (3-MA) or genetically (silencing of *ATG5*) in p53-null H1299 cells

(A549 and H460 cells are wild type in p53), an unexpected reduction of sensitivity to cisplatin was observed, again supporting a cytotoxic function of autophagy. Such observations indicate that the function of autophagy in response to cisplatin can vary among different lung cancer cell lines, acting either as a prosurvival mechanism that might mediate resistance to cisplatin, having a *non-cytoprotective* function or even contributing to cell death. Assuming that studies in cell culture have some predictive value for clinical efforts to increase patient sensitivity to therapy, these studies indicate that the observation that autophagy is induced by cisplatin in preclinical models does not necessarily predict what form the autophagy may take in the actual disease state in patients.

One potential limitation to the above-cited studies is the emphasis on cell culture and the consequent absence of experiments in tumor bearing animal models, where such factors as the role of the immune system in sensitivity to therapy with and without autophagy inhibition might have been examined.

Carboplatin is gradually replacing cisplatin in the clinic because of its lesser toxicity; however, there is little information available relating to cell death and autophagy in lung cancer cells exposed to carboplatin, although it was shown that carboplatin induces cytoprotective autophagy in other types of neoplasia such as breast³⁷ or skin cancers³⁸.

Etoposide

Etoposide is widely used for the treatment of lung cancer, frequently in combination with other cytotoxic drugs. *Kaminsky et al* showed that inhibition of autophagy induced by cisplatin and etoposide can lead to different outcomes in studies utilizing A549 and U1810 cell lines. Whereas 3-MA sensitized both cell lines to cisplatin, both by enhancing CASP-3 and PARP-1 cleavage and increasing annexin V staining, apoptosis was *attenuated* following inhibition of the autophagy induced by etoposide³⁹. While multiple explanations can likely be proposed for these observations, clearly, however, autophagy assumed different functions in the same biological system in response to different treatments. Both cisplatin and etoposide are DNA damaging agents. Cisplatin promotes DNA-DNA inter- and intrastrand crosslinks while etoposide inhibits topoisomerase II resulting in DNA double-stranded breaks^{15, 40}. The differences in the mechanisms whereby these drugs produce DNA damage and the accompanying consequences may prove to influence the nature of the resultant autophagy.

Of the multiple compounds used to sensitize drug-resistant cells to chemotherapy, Feroniellin A, a novel furanocoumarin, appears to exhibit cytotoxicity in etoposide-resistant A549 cells⁴¹. This effect was attributed to downregulation of the p-glycoprotein through the NFκB pathway and the induction of both apoptosis and autophagy. Here, interference with autophagy via siRNA-mediated downregulation of Beclin-1 was observed to decrease apoptosis, indicative of autophagy *facilitating* drug cytotoxicity.

Unfortunately, limited literature is available relating to the role of autophagy in etoposide sensitivity and resistance in non-small cell lung cancer. In small cell lung cancer, autophagy appeared to protect against both cisplatin and etoposide cytotoxicity⁴² while in leukemic cells, the opposite outcome was observed in that activation of autophagy increased

sensitivity to both etoposide and adriamycin⁴³. In several medulloblastoma cell lines, the combination of etoposide and sildenafil (utilized as a potential sensitizer to chemotherapy) resulted in different functions of autophagy in a cell line-dependent manner⁴⁴. siRNA-mediated silencing of *ATG5* and *Beclin-1* resulted in increased sensitization (by trypan blue exclusion) of HOSS1 cells to the etoposide/sildenafil combination while it decreased sensitivity to the same combination in DAOY and D283 cell lines. These findings further confirm the premise underlying this review/commentary that the nature of autophagy function in response to chemotherapy cannot be predicted, making it difficult to propose a uniform strategy of autophagy inhibition for chemosensitization.

Paclitaxel

Paclitaxel is also among the most widely used drugs in the treatment of non-small cell lung cancer, often in combination with platinum-based compounds; however, chemoresistance is a major obstacle⁴⁵. It is not yet well-established whether paclitaxel induces autophagy in different systems and the role of autophagy in drug toxicity or development of resistance is not clear^{46, 47}. A decrease in the expression of several miRNAs has been shown to accompany the development of resistance to paclitaxel. miR-17-5p in particular is significantly down regulated, which is of potential interest as this miRNA has been reported to be capable of binding Beclin-1. Decreased expression of miR-17-5p in paclitaxel-resistant cells is associated with elevated Beclin-1 levels and increased autophagy⁴⁸. Similarly, autophagy appears to facilitate resistance to docetaxel, another antimitotic agent commonly used in the clinic as second-line therapy⁴⁹.

Gemcitabine

Gemcitabine is another important chemotherapeutic agent used in the clinic, often in combination with platinum-based compounds⁵⁰. Gemcitabine is a nucleoside analog that incorporates into the body of newly synthesized DNA and promotes apoptosis. It has been shown that gemcitabine can also sensitize lung cancer cells to radiation via promoting a shift towards apoptosis-based antitumor effects⁵¹. However, there is essentially no data on the involvement of autophagy either in mediating gemcitabine action or conferring resistance to this agent.

Erlotinib and gefitinib

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors such as erlotinib and gefitinib are now classic examples of targeted cancer therapy⁵². These agents have demonstrated efficacy in the treatment of late stage non-small cell lung cancer with certain oncogenic mutations in EGFR. Unfortunately, several reports have suggested that tumors can acquire resistance to these drugs⁵³. Erlotinib is capable of inducing autophagy in non-small cell lung cancer harboring certain mutations in EGFR (e.g., exon 19 deletion)^{54, 55}. Inhibition of erlotinib-induced-autophagy, both pharmacologically and by genetic silencing, resulted in sensitization, suggesting the promotion of the cytoprotective form of autophagy. Likewise, erlotinib was shown to promote autophagy in non-small cell lung cancer cells with wild-type EGFR, such as the commonly studied H460 and A549 models⁵⁶, and again, inhibition of autophagy increased sensitivity to erlotinib, both in culture and *in vivo* xenograft models. Also, multiple studies have proposed autophagy as a resistance

mechanism to erlotinib and a possible target for increasing the drug's capacity for producing growth inhibition in lung cancer cells^{57–59}. In contrast, studies by *Fung et al* suggested that the *induction* of autophagy by rapamycin *enhanced* sensitivity of multiple solid tumor cell lines to erlotinib⁶⁰. A similar report by *Gorzalczany et al* has also suggested that rapamycin could act to sensitize H1299 NSCLC tumor cells to erlotinib⁶¹. However, as rapamycin may modulate additional signaling pathways and cellular responses, findings using rapamycin cannot be considered to rigorously implicate the promotion of autophagy in sensitization.

Gefitinib is also associated with the induction of autophagy both in cells with wild-type and mutant EGFR-expression⁶². Pharmacological inhibition of autophagy not only increased the cytotoxicity of gefitinib but also served to bypass resistance in NSCLC cells^{62–64}.

Consequently, autophagy appeared to be cytoprotective in the case of gefitinib. In another study involving the PC9 adenocarcinoma cell line, cisplatin and gefitinib, separately or in combination, were also shown to promote autophagy under conditions where the drugs expressed their cytotoxicity⁶⁴. Unexpectedly, when the two drugs were combined, their ability to kill the cells was reduced; however, activity was restored following inhibition of autophagy using chloroquine. Here again, autophagy appeared to be largely cytoprotective.

Work by *So et al* also suggests that autophagy plays a role in PC9 adenocarcinoma cell line sensitivity to erlotinib and gefitinib⁶⁶. CX-4945, a selective inhibitor of protein kinase 2, was able to induce autophagy in clones resistant to both drugs, which was accompanied by increased sequestration of EGFR into autophagosomes. Subsequently, inhibition of autophagy using 3-MA or *ATG7* knockdown attenuated apoptosis induced when CX-4945 was combined with erlotinib or gefitinib. Consequently, in these studies autophagy appeared to be involved in the promotion of tumor cell killing.

Crizotinib, a kinase inhibitor, was approved by the FDA in 2014 for the treatment of late stage non-small cell lung cancer cases with positive ALK⁶⁷. Crizotinib is capable of inducing autophagy in SPC-A1, A549 and H2228 cell lines⁶⁸. Autophagy promotion was confirmed by immunoblotting showing increased lipidation of LC3 and increased degradation of p62/SQSTM1. The combination of both 3-MA and chloroquine with crizotinib resulted in sensitization with or without an increase in the extent of apoptosis. Using a SPC-A1 cells xenograft model, the combination of crizotinib with hydroxychloroquine resulted in further reduction of tumor volume in comparison with crizotinib alone, confirming the cytoprotective function of crizotinib-induced autophagy. Another report indicates that autophagy was involved in the development of acquired resistance to crizotinib in the H3122CR-1 lung cancer cell line⁶⁹.

3. Radiation-induced autophagy

Radiation, another fundamental tool in the treatment of lung cancer, is also associated with the induction of robust autophagy in many tumor models²⁵. As is the case with chemotherapy, the function of autophagy in response to radiation is inconsistent. It has been described as a prosurvival mechanism in many systems despite evidence of its involvement in sensitizing cancer cells to radiation, as discussed below. Studies in our laboratory, where the function of autophagy in response to radiation was assessed in a number of cancer cell

lines, determined that the function of autophagy appeared to be dependent of the p53 status of the cells²⁵. Radiation induced cytoprotective autophagy in A549 and H460 cell lines, both of which express wild-type p53. Chloroquine was able to sensitize these cell lines to radiation based on cell viability and clonogenic survival assays. However, cells lacking functional p53, such as p53 null H1299 cells, and p53 mutant H358 cells were not sensitized to radiation upon the inhibition of autophagy. In the H1299 cells, inhibition of autophagy by chloroquine failed to sensitize the cells to radiation indicative to what we have described as “*non-cytoprotective*” autophagy. When p53 expression was induced by doxycycline, both *ATG5* silencing and treatment with chloroquine resulted in dramatic sensitization to radiation. Furthermore, induction of p53 by doxycycline resulted in increased apoptosis in response to radiation when autophagy was inhibited.

A recent report by Singh et al evaluated the response to radiation in H1299 non-small cell lung cancer cells where autophagy was genetically inhibited by the silencing of *ATG7*⁷⁰. Here, increased PARP1 cleavage and apoptosis were observed, but primarily when p18-CycE was co-expressed in the *ATG7*-silenced cells exposed to radiation. In studies where the H1299 cells were irradiated in the absence or presence of 3-MA (without modulation of p18-Cyc E levels), the enhancement of radiation sensitivity was relatively modest, which largely supports our own findings.

In the context of the above findings, it should be recognized that the relationship between apoptosis and autophagy remains quite complex as these cellular responses intersect at a number of common points, including the level of p53⁷¹. There is evidence that autophagy can function as a cytotoxic mechanism when cell death mediated through apoptosis is compromised, as may be the case when the pro-apoptotic Bax and Bak proteins are silenced^{72, 73}. This cytotoxic function of autophagy has also been described as type II cell death, characterized by vacuolar sequestration of cellular components that relies largely on lysosomal destruction⁷⁴. Other studies suggest that autophagy may function as a bridge ultimately leading to apoptosis or facilitating its occurrence, or alternatively that autophagy and apoptosis may cooperatively precipitate cell death^{75–78}.

As indicated above, one of the key players involved in the interaction between apoptosis and autophagy is the autophagy-promoting protein, Beclin-1. Autophagy induction mediated by Beclin-1 can be inhibited by its association with Bcl-2 and Bcl-xL; phosphorylation of the BH3 domain of Beclin-1 by certain tumor suppressors, such as death-associated protein kinase (DAPK), results in its dissociation from Bcl-2 and autophagy promotion⁷⁹. Consequently, it has been hypothesized that the decision as to whether the cellular fate is to be apoptosis or autophagy may be dictated by the nature of the molecular interaction between Beclin-1 and members of the Bcl-2 family⁸⁰.

With regard to the cytoprotective function of autophagy in response to radiation, autophagy seems to be essential in protecting the cell from the buildup of ROS⁸¹. Recent work by *Ko et al*⁸² appears to be of particular relevance to the cytoprotective function of autophagy, where radiosensitization occurred upon autophagy inhibition. In this study, *in vitro* gene silencing of essential autophagy players, such as *ATG5* and *Beclin-1*, in both H460 and A549 cell lines, resulted in significant decrease in cell death and clonogenicity in response to radiation

in comparison to autophagy-proficient cells. When these autophagy-deficient cells were implanted in immunocompromised (T cell-deficient) mice, sensitization to radiation was observed as in the cell culture studies. Tumor volume was measured over a period of six weeks and while radiation (8 Gy) was associated with notable reduction in tumor volume, silencing of both *Beclin-1* and *ATG5* resulted in an enhanced effect over the same time period.

Autophagy was also shown to be induced in the A549 cells (and other cell lines) following exposure to clinically-relevant hypoxia⁸³ based, in part, on a prominent increase in LC3 puncta. Hypoxia-induced autophagy appeared to confer resistance when the hypoxia was experienced by the cells prior to irradiation, as inhibition of autophagy using chloroquine resulted in radiosensitization (based on a reduction in clonogenic survival).

In contrast to these findings that generally support the cytoprotective function of radiation-induced autophagy, almost 10 years ago evidence was presented that autophagy could replace classical cell death when low-dose radiation was insufficient to produce apoptosis⁸⁴. Inhibition of essential pro-apoptotic proteins, for example, Bax and Bak, resulted not only in increased autophagy induction, but also in radiosensitization of human lung cancer cells. This *antisurvival* function of autophagy has also been implicated as the mechanism of radiosensitization by multiple agents including berberine⁸⁵, vitamin D or its analogs⁸⁶, and caspase inhibitors⁸⁷. In this context, both a cytotoxic autophagy (or autophagic cell death)⁸⁸ as well as a novel, cytostatic function of autophagy were identified; in the latter case, the promotion of autophagy in non-small cell lung cancer cells exposed to vitamin D/vitamin D analogs in combination with radiation resulted in growth arrest and impaired reproductive capacity without a significant upregulation of cell death (apoptosis and necrosis)^{86, 26}.

In HCC827 cells⁸⁹ PTEN knockdown conferred resistance to both radiation and gefitinib and was associated with decreased promotion of radiation-induced cytotoxic autophagy. Treatment with rapamycin alleviated the repression of cytotoxic autophagy to overcome PTEN knockdown-induced radioresistance. In another report, introduction of Beclin-1 resulted in enhanced autophagic cell death promoted by radiation⁹⁰. Beclin-1 was delivered by aerosol inhalation, and when combined with radiation, resulted in significant decrease of tumor progression in the lungs of K-ras^{LA1} lung cancer model mice as well as a reduction in tumor-associated angiogenesis. The complexity of these observations is highlighted by the fact that concomitant promotion of apoptosis (by ABT-737, a Bcl-2 inhibitor) and autophagy (by rapamycin) resulted in enhanced sensitivity of H460 cells to radiation both *in vitro* (decreased clonogenic survival) and *in vivo* (delayed tumor growth in a xenograft mouse model)⁹¹.

There is little information on the impact of autophagy on cellular systems subjected to combined radiation and chemotherapy. Surprisingly, despite many years of clinical utilization of the combination of radiation with chemotherapy, the molecular basis of sensitization is not well-defined. It has been reported that the combination of radiation with cisplatin resulted in increased apoptotic and autophagic cell death⁹². *Toulany et al* examined several components of the autophagy pathway and their effect on cisplatin-mediated radiosensitization using the H460 and A549 NSCLC cell lines⁹³. Interestingly, cisplatin was

able to radiosensitize H460 cells only in an apoptosis-*independent* manner. Manipulating key players in the cell survival pathway such as Ataxia-Telangiectasia Mutant (ATM) or 5'-AMP-activated protein kinase (AMPK) yielded different outcomes between the two cell lines. When Compound C, a known inhibitor of AMPK, was combined with cisplatin, only the A549 cells were radiosensitized. Furthermore, pharmacological inhibition of autophagy while cells were exposed to the combination of cisplatin and radiation did not appear to affect sensitivity to the combined treatment.

In another report, the dual inhibition of both PI3K and the mammalian Target of Rapamycin (mTOR) in cisplatin-resistant NSCLC cells enhanced radiosensitivity. Interestingly, this was accompanied by minimal caspase-3 cleavage, but significant induction of autophagy, indicating that autophagy might account for the decreased survival. However, inhibition of autophagy was not performed to confirm this assumption⁹⁴.

4. Conclusions and Overview

Many of the studies discussed in this review/commentary tend to support the more well-established role of autophagy in mediating tumor cells survival and protection against noxious stimuli such as chemotherapy and radiation. However, autophagy and collateral responses such as apoptosis and senescence occur to varying degrees depending on the type of antitumor agent utilized, the dose of the agent and the particular cell lines used for the studies, and the integrity of the molecular pathways involved in growth arrest and/or cell death⁹⁵. Furthermore, interference with any one of these responses does not inevitably dictate complementary alterations in the extent of the alternative response pathways⁹⁶. It is worth emphasizing the studies quite recently reported by *Eng et al*⁹⁸ in A549 non-small cell lung cancer cells where autophagy was inhibited by the silencing of *ATG7*. In testing more than 35 different cancer chemotherapeutic drugs as well as radiation, with very few exceptions, autophagy inhibition failed to alter sensitivity, consistent with our findings relating to the *non-cytoprotective* function of autophagy^{25, 26}.

While initial clinical trials have generated some promising outcomes⁹⁷, the role of autophagy in suppressing or maintaining tumor survival in the face of extrinsic stress is unpredictable. Both chemotherapy and radiation can promote a *non-cytoprotective* form of autophagy and autophagy can, of itself, be lethal. In either of these cases, autophagy inhibition would not provide the intended clinical advantage. Consequently, as the function of autophagy cannot currently be identified in the clinical setting, a much clearer understanding of the role of autophagy induced in response to chemotherapy and radiation in cell survival will be critical before the strategy of autophagy inhibition can be translated to the clinical care of cancer patients.

Another concern is that the effect of pharmacological inhibitors of autophagy, particularly chloroquine and hydroxychloroquine, on cell survival may be a consequence of mechanisms other than interference with autophagy^{98–100}. Consequently, efforts to develop more specific autophagy modulators that consider the role of genetic, epigenetic, transcriptional and posttranslational regulation of autophagy may prove to be more productive and predictive¹⁰¹.

There is currently extensive emphasis on the concept of personalized medicine, where therapy would be targeted based on the genetic background of a patient's tumor. We believe that a similar strategy will be required if autophagy inhibition is to enter the clinic as a viable adjunct to chemotherapy and radiation in cancer treatment. Specifically, it will be necessary to determine that a specific drug or drug combination and/or radiation actually promote the cytoprotective form of autophagy in a patient's malignancy. If the therapy-induced autophagy is, in fact, *non-cytoprotective*, then autophagy inhibition would be essentially useless. If the therapy-induced autophagy is cytotoxic, then autophagy inhibition would likely interfere with the effectiveness of treatment. Given that it is not presently feasible to identify the nature of therapy induced autophagy in the clinic, it would be premature to propose that autophagy inhibition be pursued as a clinical strategy for cancer treatment. Furthermore, due to the fact that it is likely that the autophagy promoted by chemotherapy or radiation in current clinical trials will often not be protective in function, there is a concern that false negative data will be generated suggesting that autophagy inhibition is ineffective; however, there is clearly potential for this approach to be successful in selected subsets of patients once patients can be stratified according to the nature of the induced autophagy. Figure 1 provides a diagrammatic overview of some of the major challenges faced in this field and potential strategies for addressing these challenges.

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HIGHLIGHTS

1. Autophagy induced by chemotherapy or radiation in non-small cell lung cancer can be protective, cytotoxic or nonprotective.
2. Without knowing the nature of the autophagy, it would be premature to expect that autophagy inhibition will lead to sensitization to any particular form of therapy.
3. If current clinical trials of autophagy inhibition are unsuccessful, this outcome may result in the erroneous conclusion that this approach lacks potential as a therapeutic strategy.
4. It will be necessary to identify biomarkers of cytoprotective autophagy before autophagy inhibition can be translated to the clinic for treatment of lung cancer or other malignancies.

Figure 1.
An overview and summary of some of the challenges relating to autophagy inhibition as an adjunct to current therapies and suggested strategies for addressing these challenges.